REMARKS

The Office Action of May 14, 2002 presents the examination of claims 69-108. This paper cancels claims 86, introducing that claim into claims 69 and 70. Claim 108 is also canceled, introducing that claim into claim 107. Claim 107 is further amended to correct a clear error in the relationship of nucleic acid elements. Claim 70 is amended to remove erroneous recitations of, "or functional analog thereof".

New claims 109-125 are added. The limitation of the "multiple cloning site" in claim 109 is supported by the specification at, e.g., page 5, lines 25-29 and original claim 21. The limitation upon the gene of interest in claim 110 is supported by the specification at, e.g., page 12, lines 7-14. The terms "histidine kinase ..." and "response regulator ..." in new claim 125 are supported by the specification at, e.g. page 9, lines 1-8 and Figure 5. The other limitations in the claims are the same as recitations in the previously pending claims.

Interview

An interview was held with the Examiner on July 10, 2002. The cooperation of the Examiner in advancing the prosecution of the present application is greatly appreciated.

In the interview, the Examiner indicated that she now understood that an important aspect of the invention is the

promoter illustrated in Figure 4 of the application and that transcription from such promoter is activated by the activated product of the R gene. The Examiner is reminded of Figure 5 of the application, which illustrates the promoter activation cascade.

The Office Action includes a number of erroneous statements about the invention. For example, at the middle of page 4, the Examiner refers to the genus of an IF promoter inducible by the expression product of an IF gene. This is possible, but what is described in the instant application is a promoter that is inducible by the activated product of a SakR gene (or analog thereof) (*See*, Figure 5. As another example, at the bottom of page 7, the Examiner states that it is unknown whether the SakP gene promoter is inducible by IF directly. The SakP gene promoter is inducible by the activated SakR gene product. Inducibility by IF is irrelevant. Applicants suppose that the fresh understanding of the invention by the Examiner obviates the need to address these and other erroneous statements about the invention in the Office Action.

Furthermore, the Examiner indicated that claims having the form of amended claims 69 and 107, thus reciting the structure of the promoter as a feature that defines the genus encompassed by the claim, might be allowable upon further consideration.

The Examiner also indicated that claims in which the "functional analogs" of the IF gene, the SakK gene and the SakR gene are obtained from *Lactobacillus* might be allowable. Accordingly, the Examiner's attention is directed to claim 102 and new claim 124.

Revised Sequence Listing

This Amendment is accompanied by a revised Sequence Listing. The revised sequences correct errors introduced in the Substitute Sequence Listing filed February 1, 1999, in which the sequences of Figure 4 were incorrectly shown. No new matter is introduced by the revised Sequence Listing, as the corrected sequences are shown in Figure 4.

The printed copy of the revised Sequence Listing is identical to the CRF copy provided on the attached diskette in the file 1380-0122P.ST25.txt, except that the CRF copy lacks formatting information.

Rejections under 35 U.S.C. § 112, first paragraph

Written Description

Claims 69-108 were rejected under 35 U.S.C. § 112, first paragraph, for allegedly lack of adequate written description support for the claimed invention in the specification. This

rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

First, the Examiner asserts that the promoter recited in the claims (taking claim 69 as representative) is described only by its function, i.e. as activated by an activated product of an R gene. As discussed in the interview, claims 69 (and 70) have been amended to incorporate structural limitations on the promoter sequence that is activated by the activated R gene product. These structural limitations on the promoter are a "common structural feature" that serves to define the generic invention. The Examiner should note page 17, lines 26-27 ("conserved region of promoter depicted in Fig. 4") and page 18, lines 1-4 ("common characteristics in regulable promoters that are indicated in Fig. 4").

Furthermore, as to claims 96, 97 and new claims 119 and 120, the Examiner should note that the IF peptide is recited as a particular amino acid sequence. This is a further structural feature that defines this subgeneric invention.

In the interview of July 10, 2002, the Examiner expressed a concern that the nucleotide sequences of the SakK and SakR genes, and of analogs thereof, are not described in the present specification. The Examiner should consider that a specification need not teach, and preferably omits, that which is known in the art. *Spectra-Physics, Inc. v. Choherent, Inc.* 3

USPQ2d 1737, 1743 (Fed. Cir. 1987). The written description must show that the inventors had the claimed invention in their possession at the time of filing of the application. *See, e.g. Vas-Cath, Inc. v. Mahurkar* 19 USPQ2d 1111, 1116 (Fed. Cir. 1991).

The Examiner is reminded that the nucleotide sequences of these genes were known in the art at the time the present application was filed. The Examiner is referred to page 3, lines 24-29 of the specification, where reference is made to the EMBL/GenBank/DDBJ database accession number Z48542 as describing the genes from two isolates of *Lactobacillus sake* that encode and control the production of the bacteriocin sakacin P. As to functional analogs, the Examiner should also note, for instance, to Axelsson et al., *J. Bacteriol.* 177:2125-2137 (1995) (of record by IDS of 8/11/98), which describes the sequences of the SapK and SapR genes of *Lactobacillus sake.* As mentioned in Applicants' previous response, analogs of the SakK and SakR genes are described in another publication, e.g. Diep et al., *Applied and Environmental Microbiology,* 60:160-166 (1994) provide the nucleotide sequences of the *pln*A (IF analog) and *pln*BCD (SakK, SakR analog) genes of *Lactobacillus plantarum* (see Fig. 1). As nucleotide sequences of the genes set forth in the claims were known at the time of filing of the application, it

is not necessary that these sequences be set forth in the instant specification.

The sequence of the IF peptide is set forth in the present application. A nucleotide sequence that would encode the IF peptide is also provided in the Figures of the present application.

As relevant sequences of the genes recited in the claims are either described in the specification or in the prior art, there is description of these items sufficient to demonstrate possession of these elements of the invention by the inventors at the time of filing of the application. Thus, there is adequate written description support for these aspects of the present claims.

The Examiner also erroneously believes that the number of representative species of promoters of the invention disclosed in the specification is one. (Page 4, line 9 of the Office Action.) In fact, five promoters having the requisite properties to function in the invention are set forth in Figure 4 of the application and in the Sequence Listing (SEQ ID NOS: 6-10). Thus, the number of species of the claimed promoter described in the specification is five, and the number of species of IF, SaK, SakR or analogs within the scope of the claims that are described is two.

Enablement

Claims 69-108 stand rejected under 35 U.S.C. § 112, first paragraph, for alleged lack of enabling disclosure of the claimed invention. This rejection is respectfully traversed. Reconsideration and withdrawal thereof are requested.

Applicants have previously provided their arguments on this issue. These arguments are repeated here for convenient review by the Examiner:

> As a threshold matter, the Examiner fails to establish a proper *prima facie* case for lack of enablement. The Examiner merely states in summary fashion that the specification does not reasonably provide enablement for an expression system comprising functional analogs of [IF, SakK and SakR genes and their products] nor of a method for their use.
>
> Once again, the issue in determining whether a specification enables a claimed invention is whether undue experimentation is required to practice the invention throughout the scope of the claims. The amount of experimentation is not determinative, but is only one factor to consider. The nature of the invention, the breadth of the claims, the guidance provided by the specification, the level of skill in the art, the presence or absence of working examples, the prior knowledge of the skilled artisan and the predictability of the art must all be considered. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

In the present instance, the invention described in the rejected claims lies in a gene expression system comprising as its recited elements three genes and a promoter from which transcription of a desired nucleic acid is effected, wherein the products of the three genes activate one another, finally activating the promoter. The Examiner's objection lies in the

breadth of the claims, which recite "functional analogs" of the IF, SakK and SakR genes expressly described in the application. The level of skill in the art of biotechnology is generally accepted to be very high. Most practitioners in the field hold advanced degrees; very many hold doctoral degrees and have several years of laboratory experience.

The knowledge in the art of "two-component" regulatory systems, of which the present invention is an example, is fairly high, as evidenced by the citation by the Examiner of four references said to anticipate the present invention. For example, there was extensive knowledge about how the genes regulating nisin expression function at the time the invention was made. Practitioners implementing further embodiments of the present invention, beyond those specifically exemplified in the present specification, may draw upon that knowledge.

The guidance provided by the specification is extensive. That guidance includes a description of an assay for determining whether a given bacterial type expresses a set of genes providing a regulatory phenotype similar to that provided by the IF-K-R complex (Example 1). This assay was applied to characterize and isolate the IF-K-R complex from *Lactobacillus sake* and also a second complex of genes from *Lactobacillus plantarum* C11. Thus, there are two working examples of the invention provided by the specification. Assays for function of

IF peptides are also described (Examples 2 and 4). The specification further informs the practitioner that functional variants are likely to be found in bacteria of the genus *Lactobacillus*. (*See*, page 10, lines 1-5 and line 18.) Thus, the skilled artisan is provided with guidance as to where to start looking for functional variants if IF, SakK and SakR genes. Example 6 shows that this guidance is effective.

The Examiner's primary point seems to be that the art is unpredictable. Applicants have previously acknowledged that is the case, but again, the sort of unpredictability asserted by the Examiner is not determinative.

Applicants agree that, *a priori* one of skill cannot tell if a set of genes will have the properties described for "functional analogs" of the IF, SakK and SakR genes. In particular, if they would function to control a promoter upon activation of a cascade started by an "IF" peptide is not apparent merely by examination of a gene sequence. However, examination of the organization of a set of genes can provide helpful clues. *See*, page 10, lines 1-5 of the specification. Furthermore, the specification informs the skilled artisan how to determine whether a given set of genes represents a functional analog of the IF, SakK and SakR genes and also provides guidance as to where to begin to search for such functional analogs.

The Court of Appeals for the Federal Circuit, in the *Wands* case, expressly held that experimentation considered typical in an art is not undue. In *Wands*, a hybridoma invention claimed in broad, functional terms was considered well-enabled, despite the facts that (1) only 2.8% of hybridomas screened produced a functional antibody and (2) that only 2 of 10 screening experiments succeeded at all.

The skilled artisan in molecular biology, especially in the art of gene expression, expects to have to perform various screening experiments to isolate functional variants of already known genes and their products. Such screening is manifestly not undue experimentation under the holding of *In re Wands*.

Applicants submit that, in view of the above considerations, it is not undue experimentation to practice the invention as claimed. Accordingly the instant rejection should not be applied to the present claims.

Applicants further note that evidence of record, in particular Exhibits 1-4 filed with and explained in Applicants response of October 16, 2000, shows that "functional analogs" of the IF, SakK and SakR genes and their products can be isolated following the teachings of the specification. The Examiner has never addressed this evidence or Applicants' explanation of it.

Applicants have also noted the Examiner's comment at page 6, lines 14-15 of the previous (Oct. 2, 2001) Office Action that,

"the claims are not directed to structurally homologous genes from *Lactobacillus*." Claim 77 and new claims 112-119 recite that the functional analogs are from a lactic acid bacterium and thus at least this claim should be considered free of this rejection.

Applicants respectfully point out again that there is already evidence of record (Exhibits 1-4) that establishes that one of ordinary skill in the art, following the teachings of the specification, can make further embodiments of the invention. Applicants submit that this evidence is sufficient basis for withdrawal of the instant rejection.

Applicants address some additional points raised by the Examiner below.

At page 9, the Examiner states that the specification does not teach any promoter, other than the IF promoter, from which the SakK and SakR genes can be expressed. Applicants submit that a number of promoters useful for expression of genes in bacteria were known in the art at the time the present application was filed. The Examiner should note that the present claims describe arrangements of the IF, SakK and SakR genes that differ from the arrangement of an operon driven by the IF promoter that is found in nature. The Examiner also seems to question whether the present system would work in a host other than *Lactobacillus*. Applicants submit that the

specification makes clear that the presence of polypeptide products of only three genes, IF, SakK and SakR, are necessary for operability of the invention. The Examiner provides no reasoning or evidence that expression of genes of one bacteria in a second kind of bacteria is at all difficult and so there is no *prima facie* lack of enablement of this aspect of the invention. Furthermore, some claims, e.g. claims 80, 113 and 123 among others, recite a lactic acid bacterial host cell.

At page 11, the Examiner asserts that the specification fails to teach "the structural requirements for the sequence to impart the requisite function (of inducing the SakP promoter). This is incorrect. The Examiner is referred to Figure 4, which clearly discloses the conserved sequences that are alleged to confer this "requisite function" and are recited in the claims.

For all of the above reasons, Applicants submit that the disclosure of the specification is fully enabling of practice of the claimed invention. Accordingly, the instant rejection should be withdrawn.

For all of the above reasons, Applicants respectfully submit that all of the present claims define patentable subject matter such that this application should be placed into condition for allowance. Early and favorable action of the merits of the present application is respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Mark J. Nuell (Reg. No. 36,623) at the telephone number below, to discuss such matters.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a two (2) month extension of time for filing a reply in connection with the present application, and the required fee of $200.00 is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and further replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fee required under 37 C.F.R. 1.16 or under 37 C.F.R. 1.17; particularly, extension of time fees.

Respectfully yours,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claims 86 and 108 have been canceled.

The claims have been amended as follows:

69. (amended) A gene expression system comprising:

(a) an IF gene, or a functional analogue thereof;

(b) a SakK gene, or a functional analogue thereof;

(c) a SakR gene, or a functional analogue thereof;

(d) a cloned polynucleotide of interest linked to a first inducible promoter,

wherein in said gene expression system, the expression product of the IF gene, or functional analogue thereof

(i) induces the production of bacteriocins by a lactic acid bacterium,

(ii) is not a lantibiotic, and

(iii) induces the expression of genes regulating bacteriocin production in said lactic acid bacterium, and

(iv) activates the expression product of the SakK gene, or functional analogue thereof, and

the activated expression product of the SakK gene, or functional analogue thereof, activates the expression product of the SakR gene, or functional analogue thereof, and

the activated expression product of the SakR gene, or functional analogue thereof, induces the first inducible promoter of the gene of interest,

thereby causing expression of the gene of interest; and

wherein the IF gene or functional analogue thereof is expressed from a promoter different from the promoter from which the SakK gene or functional analogue thereof and/or the SakR gene or functional analogue thereof are expressed; and

wherein the first inducible promoter comprises two repeated nucleotide sequences 5 to 10 nucleotides long and spaced 17 to 23 nucleotides apart, wherein the downstream member of said repeated sequence is located 30 to 38 nucleotides upstream from a -10 region of a bacterial gene, and wherein said repeated nucleotide sequences are selected from the group consisting of residues 7-14 and 30-38 of SEQ ID NO:6, residues 7-14 and 30-38 of SEQ ID NO:7, residues 7-14 and 30-38 of SEQ ID NO:8, residues 7-14 and 31-38 of SEQ ID NO:9, and residues 7-8, 10-14 and 31-38 of SEQ ID NO:10.

70. (amended) A gene expression system comprising:

(a) an IF gene;

(b) a SakK gene;

(c) a SakR gene;

(d) a cloned polynucleotide of interest linked to a first inducible promoter,

wherein in said gene expression system, the expression product of the IF gene activates the expression product of the SakK gene, and

the activated expression product of the SakK gene activates the expression product of the SakR gene and

the activated expression product of the SakR gene induces the first inducible promoter of the gene of interest,

thereby causing expression of the gene of interest;

wherein said the expression product of said IF gene is not a lantibiotic; and

wherein the IF gene [or functional analogue thereof] is expressed from a promoter different from the promoter from which the SakK gene [or functional analogue thereof] and/or the SakR gene [or functional analogue thereof] are expressed; and

wherein the first inducible promoter comprises two repeated nucleotide sequences 5 to 10 nucleotides long and spaced 17 to 23 nucleotides apart, wherein the downstream member of said repeated sequence is located 30 to 38 nucleotides upstream from a -10 region of a bacterial gene, and wherein said repeated nucleotide sequences are selected from the group consisting of residues 7-14 and 30-38 of SEQ ID NO:6, residues 7-14 and 30-38 of SEQ ID NO:7, residues 7-14 and 30-38 of SEQ ID NO:8, residues

7-14 and 31-38 of SEQ ID NO:9, and residues 7-8, 10-14 and 31-38 of SEQ ID NO:10.

107. (amended) An isolated nucleic acid comprising:

two repeated nucleotide sequences 5 to 10 nucleotides long and spaced 17 to 23 nucleotides apart, wherein the downstream member of said repeated sequence is located 30 to 38 nucleotides [downstream] upstream from a

-10 region of a bacterial gene,

wherein transcription of a coding nucleic acid sequence operatively linked to said isolated nucleic acid is activated by an expression product of a SakR gene or functional analog thereof that has been activated by an expression product of a SakK gene or functional analog thereof, wherein said repeated nucleotide sequences are selected from the group consisting of residues 7-14 and 30-38 of SEQ ID NO:6, residues 7-14 and 30-38 of SEQ ID NO:7, residues 7-14 and 30-38 of SEQ ID NO:8, residues 7-14 and 31-38 of SEQ ID NO:9, residues 7-8, 10-14 and 31-38 of SEQ ID NO:10.

Claims 109-125 have been added.